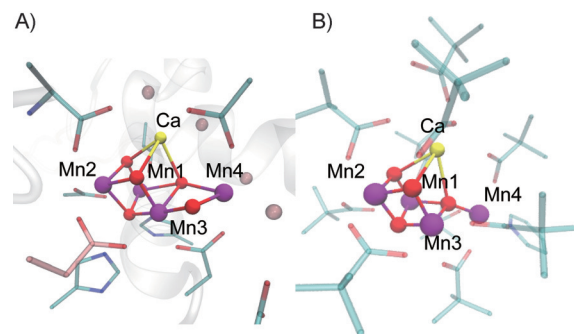


# Synthetic Model of the Oxygen-Evolving Center: Photosystem II under the Spotlight

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The oxygen-evolving center (OEC) in photosystem II catalyzes a water splitting reaction. Great efforts have already been made to artificially synthesize the OEC, in order to elucidate the structure-function relationship and the mechanism of the reaction. Now, a new synthetic model makes the best mimic yet of the OEC. This recent study opens up the possibility to study the mechanism of photosystem II and photosynthesis in general for applications in renewable energy and synthetic biology.



**Figure 1.** A) Comparison of the native OEC<sup>[6]</sup> (PDB ID: 3WU23, rendered with VMD) and B) its synthetic model<sup>[8]</sup> (Cambridge Crystallographic Database deposit number: 1042697, rendered with VMD).

Water splitting takes place at the oxygen-evolving center (OEC) of photosystem II in plants, algae, and cyanobacteria. During the reaction, oxygen atoms in water are oxidized to O<sub>2</sub> while protons and electrons are used to convert light energy into chemical energy. To oxidize water, the OEC operates at a redox potential of ~1 V, one of the highest redox potentials in biological systems.<sup>[1]</sup> Yet it achieves amazing efficiency with an estimated turnover frequency of 40 s<sup>-1</sup> and a turnover number of 600 000.<sup>[2]</sup> Because it is the only catalyst for biological water oxidation, the OEC has been the source of O<sub>2</sub> production for the past 3 billion years, shaping the whole biosphere. Due to its efficiency and robustness, the OEC could provide important design principles for artificial water-oxidation catalysis for renewable energy applications.

The OEC is a heteronuclear metal cluster composed of Mn, Ca, and O atoms. Along with the FeMoCo core in nitrogenase enzymes and the Ni-Fe cofactor in carbon monoxide dehydrogenase,<sup>[3,4,16]</sup> the OEC is among the most complex metal cofactors in biomolecules. Before high-resolution crystal structures were available, X-ray absorption spectroscopy studies identified the OEC as an oxo-bridged metal cluster with four Mn<sup>n+</sup> ions and one Ca<sup>2+</sup> ion and provided several possible structures of the OEC.<sup>[5]</sup> A 1.9 Å resolution X-ray structure of photosystem II has revealed its unique active site at resting state, which harbors a Mn<sub>3</sub>O<sub>4</sub>Ca cubane structure and a dangling Mn ion, resembling a distorted chair (Figure 1 A).<sup>[6]</sup> This crystal structure

of the OEC also agrees with previous XAS data and other spectroscopic data.

During its photocatalytic cycle, the OEC can go from S<sub>0</sub> to S<sub>4</sub> state, with S<sub>0</sub> being the most reduced form (having 3 Mn<sup>3+</sup> ions and 1 Mn<sup>4+</sup> ion). Although numerous computational and spectroscopic studies have shed light on the reaction mechanism of water splitting and the intermediate structure of the OEC, many questions remain to be answered: including the structure and ligand environment of the more oxidized intermediates and redox potentials between various oxidation states.

Because the OEC resides in a 350 kDa membrane-protein complex, it is challenging to study the reaction mechanism and redox chemistry of the OEC directly. Synthetic model complexes that mimic both the structure and function of the OEC provide a facile strategy to capture the reaction intermediates, characterize their structural, spectroscopic, and redox properties, and calibrate computational studies. There are a few Mn-based functional models of the OEC that can oxidize water,<sup>[2,20]</sup> but their structures are distinctly different from that of the natural OEC. With an asymmetric structure and several high-valent metal ions, the synthesis of a functional OEC model is highly challenging for inorganic chemists. In 2011, Kanady et al. reported the synthesis of a Mn<sub>3</sub>O<sub>4</sub>Ca cluster, which is structurally similar to the cubane part of the OEC.<sup>[7]</sup> Mukherjee et al. reported a closer mimic, which contains a Mn<sub>3</sub>O<sub>4</sub>Ca cubane with a dangling Ca atom.<sup>[17]</sup> These synthetic models provide information about the role of calcium and the redox chemistry of the cluster.<sup>[18,19]</sup> Recently, a Mn<sub>4</sub>Ca model compound of the OEC (Figure 1 B) was reported by Zhang et al.<sup>[8]</sup> In addition to the Mn<sub>3</sub>O<sub>4</sub>Ca cubane, this model includes the “dangler” Mn

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ion, and the Mn–Mn distances match those from the native cluster, making it the best OEC structural model yet available.<sup>[8]</sup>

Besides structural similarity, the new Mn<sub>4</sub>Ca model features complex electrochemical behavior and, upon one-electron oxidation, was capable of reproducing spectroscopic features reminiscent of the S<sub>2</sub> state from the native enzyme. In the cyclic voltammogram, four redox processes were accessible, although not all reversible or in a physiologically relevant range, which indicates that the complex can accumulate oxidation equivalents in a manner similar to the transitions between S<sub>0</sub>–S<sub>4</sub> states in the native OEC. To further demonstrate its similarity to the native cluster, the S<sub>2</sub> state of the synthetic model was trapped by using a chemical oxidant with the appropriate oxidation potential. The authors then performed extensive electron paramagnetic resonance (EPR) spectroscopy on the synthetic model in the S<sub>2</sub> state. The two signals in the EPR spectrum, one with  $g=4.9$  and one with  $g=2.0$ , are similar to those from the native OEC in the S<sub>2</sub> state.<sup>[9,10]</sup> Such characteristic signals have never been observed in other synthetic models. Additionally, the ligands at the Mn4 and Ca positions could be exchanged by alternative neutral ligands, which indicated that they are possible binding sites for the water molecule. The resemblance between the synthetic Mn<sub>4</sub>Ca cluster and the native OEC suggests that this synthetic model is a good starting point to study the mechanism of the water-splitting reaction in great depth. With the structural and spectroscopic resemblances, a natural question to ask is whether the synthetic model could perform water-oxidation chemistry. Zhang et al. did not mention that this had been tested.<sup>[8]</sup>

Because the synthetic model has similar structural and spectroscopic features (for the S<sub>2</sub> intermediate) to the native cluster, its redox behavior could provide insight about the redox chemistry of the OEC in photosystem II. By correlating its physical properties, such as magnetic behavior and structure, with its chemical properties, such as redox state and potential, further information could be gathered for water oxidation by the native OEC and artificial systems.

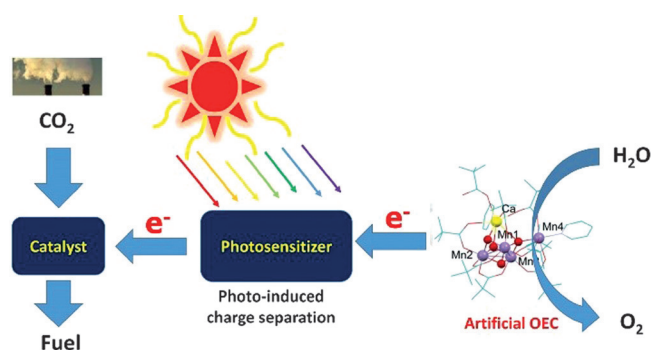
Despite the structural resemblance and functional implications, there are still opportunities to produce better OEC model complexes. Compared to the native OEC, the current synthetic Mn<sub>4</sub>Ca cluster still lacks one O ligand connecting the “dangler” Mn ion with a Mn ion in the cubane. Additionally, the distances between Mn1 and O5 (O atom between Mn1, Mn3, Mn4, and Ca) are quite different between the synthetic complex and the native OEC, although a long Mn1–O5 distance is crucial for water coordination to Mn1. Another challenge for the development of a functional model of the OEC is the direct observation of water oxidation, which has not yet been shown.

By increasing the mechanistic understanding of photosystem II, the next question that can be asked is whether it is possible to construct artificial photosynthetic systems *in vitro* and *in vivo*. One of the main goals of this would be to build an artificial photosynthetic apparatus from scratch. Great progress has been made in this field by using heterogeneous metal-oxide catalysts.<sup>[11]</sup> Among them, some can even achieve an efficiency higher than photosynthesis.<sup>[21]</sup> While H<sub>2</sub> is the

most common product and electron sink for water oxidation, electrons from the reaction could also be used as the source for CO<sub>2</sub> reduction, generating carbon-based fuels and fixing CO<sub>2</sub> at the same time.<sup>[22]</sup>

While some synthetic inorganic compounds are already powerful water-oxidation catalysts, nature wraps its catalyst with protein. In native photosystem II, the MnCa cluster is coordinated by seven amino acid residues (Asp, Glu, Ala, His), and its second coordination sphere includes three additional residues (Asp, Arg, Glu).<sup>[6]</sup> These residues help to stabilize the MnCa cluster by limiting the interaction between the catalytic core and the water molecules, and preventing the metal core from collapsing or aggregating. To develop useful catalysts for water splitting, the robustness of the artificial OEC can be improved by providing an appropriate macromolecule scaffold or polymer matrix to support the metal cluster.<sup>[12,13]</sup>

For that purpose, small protein models have been proven to be valuable for studying large, complex metalloenzymes.<sup>[14,15]</sup> While the synthetic chemistry approach allows for atomic control and accurate recapitulation of the coordination shell, it remains difficult to install secondary shell residues, which are essential for optimizing proton and electron transfer. Using molecular biology techniques and expansion of the genetic code, small protein models of complex metalloenzymes can be constructed. Through this approach, an artificial OEC model, proton and electron relays, photosensitizers, and CO<sub>2</sub>-reducing catalysts might be assembled optimally, resulting in a highly



**Figure 2.** An integrative system for the conversion of CO<sub>2</sub> into fuel by using the electrons provided by the artificial OEC through water splitting.

efficient catalytic system to convert CO<sub>2</sub> into fuel by using the electrons and protons provided by water splitting (Figure 2).

The work of Zhang et al. is an important milestone in photosynthesis research and has many implications in synthetic biology and renewable energy. One of the most important properties of photosystem II is that it is capable of using water as an electron source for CO<sub>2</sub> reduction. However, the reducing electrons have to be relayed through a complex system to produce NADPH, and then NADPH is used for CO<sub>2</sub> reduction (also through a complex, and inefficient cycle, called the Calvin cycle). By using a simplified model of the OEC, the reducing electrons provided by water splitting could be much more easily harnessed to drive a large number of chemical reactions.

This new synthetic OEC model opens up many possibilities to study the mechanism of water splitting, as well as developing an inexpensive catalyst with an earth-abundant source for artificial photosynthesis.

**Keywords:** metal clusters • oxygen-evolving centers • photosynthesis • photosystem II • water splitting

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